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CONTROLLED RELEASE FORMULATION COMPRISING GnRH-II

FIELD OF INVENTION

The present invention relates to a pharmaceutical preparation that releases a therapeutic agent over an extended period.

BACKGROUND TO THE INVENTION

Studies on the physiology of the hypothalamic-pituitary-gonadal axis have resulted in the recognition of gonadotropin releasing hormone (GnRH, otherwise known as luteinizing hormone releasing hormone, LHRH) as a key regulatory hormone. GnRH is released by the hypothalamus and acts on the pituitary to stimulate the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). More recently, a peptide with homology to GnRH has been identified (White *et al.*, Proc. Natl. Acad. Sci. USA 95 305-309, 1998). This peptide has been called GnRH-II. The sequences of the two peptides are compared below.

GnRH      pyroGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH<sub>2</sub> (SEQ I.D. No.5)  
GnRH-II   pyroGlu-His-Trp-Ser-His-Gly-Trp-Tyr-Pro-Gly-NH<sub>2</sub> (SEQ I.D. No.6)

The name "GnRH-II" is, to some extent, misleading. The new peptide is a separate gene product, and is clearly distinguishable from GnRH in its tissue distribution. It seems unlikely that GnRH-II acts as an endogenous releaser of LH and FSH. Since no clear evidence for a physiological role for GnRH-II has been presented, no attention has been paid to the practical aspects of using this peptide as a therapeutic agent.

SUMMARY OF THE INVENTION

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We have now found that GnRH-II has an important role in the function of a number of organs. For example, it influences osteogenesis and it modulates the proliferation of prostatic epithelial cells. Accordingly, we have considered the means by which this agent and its analogues might usefully be delivered in a clinical situation, and it is an object of the present invention to provide suitable formulations for achieving this purpose. The formulations according to the present invention rely on the use of a

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biodegradable polymer to hold the peptide in a depot, from which it is released into the systemic circulation at a controlled rate. These formulations comprise two key elements, the biologically active peptide and the biodegradable polymer. The biologically active peptide is a decapeptide according to the sequence

pyroGlu-His-Trp-Ser-Xaa<sup>1</sup>-Gly-Xaa<sup>2</sup>-Xaa<sup>3</sup>-Pro-Gly-NH<sub>2</sub> (SEQ. I.D. No.7)

wherein Xaa<sup>1</sup> is His or Tyr,  
Xaa<sup>2</sup> is Trp or Leu, and  
Xaa<sup>3</sup> is Tyr or Arg,

provided that when Xaa<sup>1</sup> is Tyr and Xaa<sup>2</sup> is Leu, then Xaa<sup>3</sup> is not Arg. The polymer is any pharmaceutically acceptable biodegradable polymer, and preferably a co-polymer of glycolic and lactic acids. The invention further comprises the use of the formulations for the treatment of human pathologies.

#### DESCRIPTION OF THE FIGURE

Figure 1 shows the effect of increasing doses of GnRH-II on serum calcium concentrations in ovariectomised rats.

#### DESCRIPTION OF THE INVENTION

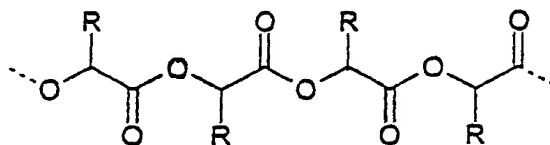
As used herein, abbreviations referring to amino acids have their conventional meanings and indicate the natural L-isomer (except for the achiral amino acid glycine).

In a first aspect, the invention as disclosed herein comprises a pharmaceutical formulation that releases a therapeutic peptide at a controlled rate and for an extended period of time (i.e. for a period of at least one day, preferably several days, and more preferably at least one week), particularly for the treatment of diseases of the bone and prostate. The therapeutic peptide is a decapeptide according to the sequence

pyroGlu-His-Trp-Ser-Xaa<sup>1</sup>-Gly-Xaa<sup>2</sup>-Xaa<sup>3</sup>-Pro-Gly-NH<sub>2</sub> (7)

wherein Xaa<sup>1</sup> is either His or Tyr, Xaa<sup>2</sup> is either Trp or Leu, and Xaa<sup>3</sup> is either Tyr or Arg, provided that when Xaa<sup>1</sup> is Tyr and Xaa<sup>2</sup> is Leu, then Xaa<sup>3</sup> is not Arg. Preferably, Xaa<sup>1</sup> is His, Xaa<sup>2</sup> is Trp, and Xaa<sup>3</sup> is Tyr. It will be recognised that such a peptide can form salts with acids (for example, acetic acid, trifluoroacetic acid, benzoic acid, hydrochloric acid, phosphoric acid and the like). To the extent that such salts are formed with pharmaceutically acceptable acids, they are included within the scope of the invention.

A second essential component of the formulation is a biodegradable, pharmaceutically acceptable polymer. Such polymers are known in the art. They can either be homopolymers (i.e. polymers of a single monomer) or copolymers (i.e. formed from two or more different monomers). Suitable monomers include amino and hydroxy derivatives of carboxylic acids. In a preferred embodiment of the present invention, the polymer is composed of hydroxyacyl monomeric units, and more preferably of  $\alpha$ -hydroxyacyl units. Most preferably, the polymer is a poly(glycolic acid), a poly(lactic acid) or a copolymer of glycolic and lactic acids. Such a polymer has the following chemical structure.



where R is hydrogen in poly(glycolic acid), methyl in poly(lactic acid), and randomly hydrogen or methyl in the copolymer.

Two complementary methods for making the formulation of the present invention can be distinguished. The peptide can either be incorporated into a matrix of the polymer, or, more preferably, it can be encapsulated by the polymer. In this second case, the peptide that is encapsulated may be either a solid or in solution. It is preferred for the peptide to be a solid.

This formulation is useful in the treatment of human pathologies, including disorders of bone growth (including age-related osteoporosis and osteoporosis associated with post-menopausal hormone status, primary and secondary hyperparathyroidism, disuse osteoporosis, diabetes-related osteoporosis, and glucocorticoid-related

osteoporosis) and prostate growth (including benign prostatic hyperplasia and prostate cancer).

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In a second aspect, the invention as disclosed herein comprises a method for the treatment of an individual suffering from a disorder of bone or prostate growth, or considered to be at risk of so suffering. This method of treatment comprises the administration to said individual of a therapeutically effective amount of a formulation containing, as an active principal, a peptide according to the sequence



or a pharmaceutically acceptable salt thereof, wherein Xaa<sup>1</sup>, Xaa<sup>2</sup> and Xaa<sup>3</sup> are as defined above, and as a second component, a pharmaceutically acceptable biodegradable polymer, which formulation releases the peptide into the systemic circulation as the polymer is eroded. The method of treatment may comprise a single administration of the formulation, but is more likely to comprise a course of repeated administrations. The frequency of the administrations may be from once per day to once per month. The amount of active peptide in each dose will depend on the dosing schedule and the route of administration. Generally, it will be between one milligram (1mg) and one gram (1g). The supervising physician will determine the precise dose depending on the parameters generally considered in the art to be relevant. The formulation is administered by intramuscular or subcutaneous injection.

The peptides that comprise the active agents of the compositions of the present invention can be prepared by the methods generally known in the art. For example, the peptides may be prepared by solid-phase synthesis. This involves the sequential addition of amino acid residues to a resin-bound intermediate according to the following strategy.

1. Formation of resin-bound first intermediate
  - PG-Aaa-OH + FG-Res -> PG-Aaa-L-Res
  - Aaa = amino acid
  - PG = protecting group
  - FG = functional group
  - Res = polymeric resin

L = linker group ( -O- or -NH- )

2. Deprotection

PG-Aaa-L-Res - H-Aaa-L-Res

3. Chain extension

PG-Bbb-OH + H-Aaa-L-Res - PG-Bbb-Aaa-L-Res

4. Repeat steps 2 and 3 as necessary

PG-Bbb-Aaa-L-Res - - - PG-Nnn-...-Bbb-Aaa-L-Res

5. Cleave/deprotect

PG-Nnn-...-Bbb-Aaa-L-Res - H-Nnn-...-Bbb-Aaa-OH (or -NH<sub>2</sub>)

In step one, a protected amino acid is reacted with a functionalised resin. The protecting group (PG) is most commonly *tert*-butoxycarbonyl (Boc) or 9-fluorenylmethyloxycarbonyl (Fmoc). The functional group on the resin (FG) is commonly a chloroalkyl group, a hydroxyl group or an amine group. When FG is a chloroalkyl or hydroxyl group, the linker group (L) is an oxygen atom ( -O- ). When FG is an amine group, L is -NH-.

In step two, the protecting group (PG) is removed from the  $\alpha$ -amino group. When PG is Boc, this can be accomplished by treating the resin with acids such as trifluoroacetic acid or hydrogen chloride in dichloromethane. When PG is Fmoc, the deprotection can be accomplished by treating the resin with bases such as piperidine.

In step three, the peptide chain is extended by one amino acid residue. A protected amino acid is coupled to the amine group liberated in step two. Many reagents are known in the art for achieving this conversion. One combination is dicyclohexylcarbodiimide (DCC) and hydroxybenzotriazole (HOBt). Generally, a base will also be necessary. Suitable bases include triethylamine and *N,N*-diisopropylethylamine. The solvent will generally be dichloromethane, dimethylformamide, or a mixture of these.

If the side chains of the amino acids (Aaa - Nnn) contain reactive groups (for example amino groups, carboxylic acid groups, hydroxyl groups) then these will need protecting. The protecting groups chosen for the side chains are generally those that are stable under the conditions required to remove the protecting group (PG) from the  $\alpha$ -amino group. If PG is Fmoc, then the side chain protecting groups can conveniently be based on tert-butyl chemistry. On the other hand, if PG is Boc, then the side chain protecting groups can be based on fluorenylmethyl chemistry. Other protecting groups known in the art can also be used.

In step four, the deprotection/chain extension cycle is repeated until the desired peptide sequence has been constructed.

In step five, the completed peptide is liberated from the resin. Protecting groups are removed from the side chains either before or after the cleavage. When L is -NH-, the peptide liberated is in the form of the C-terminal amide. When L is -O-, the peptide liberated is often the C-terminal free acid and a second step is required to form the C-terminal amide.

The peptides may also be prepared by solution-phase synthesis, and this may be more convenient when large quantities of material are needed.

The polymers required for the formulation are generally well known in the art. As stated previously, the formulation may take the form of a simple dispersion of the peptide in a matrix of the polymer, or the peptide may be microencapsulated with the polymer. Dispersions can be prepared by mixing the peptide (as a solid) and the polymer to homogeneity, then compressing the mixture to form a solid mass. It may be necessary to add a binding agent to the mixture in order to achieve a suitably cohesive composition. The mass can then be ground up to give particles suitable for suspension in a biologically compatible liquid (such as water or isotonic saline) and injection.

Microencapsulated formulations can be prepared either from the solid peptide (as a powder) or from a solution, and particularly an aqueous solution, of the peptide. The polymer is first dissolved in a suitable organic solvent. The peptide is then added to this solution and the mixture is vigorously stirred to disperse the peptide in the organic phase. A second organic solvent is then added. This second solvent is

chosen to reduce the solubility of the polymer in the organic phase. The polymer comes out of solution to form a coating around the particles of solid peptide (or around the droplets of dispersed aqueous solution). The resultant microcapsules are then hardened by washing to remove traces of the organic solvents. They are then ready to be suspended in an appropriate liquid for administration.

The above general description is further elaborated below in a number of examples.

These are intended to illustrate certain aspects of the invention. They are not intended to be limiting in any way.

## EXAMPLES

### Example 1 - Synthesis of GnRH-II

### 1A. Preparation of resin-bound protected peptide.

pyroGlu-His(Bom)-Trp(CHO)-Ser(Bzl)-His(Bom)-Gly-Trp(CHO)-Tyr(Bzl)-Pro-Gly-Ores

This peptide was prepared using standard solid-phase methods starting from Boc-Gly-esterified Merrifield resin (60 g, 1 mmol/g). The synthesis was performed in a manual synthesizer, with a total solvent and reagent volume of 300 mL for each operation. The standard deprotection/wash/coupling protocol is summarised in Table 1.

Table 1

Step	Reagent	Time (min)	Number of Operations
Deprotection of Boc	HCl/DCM*	60	1
Washing	DCM	2 - 4	3
Neutralisation	10% DIPEA/DCM	4	2
Washing	DCM	2 - 4	1
Coupling	Activated ester	60 - 120**	1 - 2
Washing	DCM	2 - 4	3
* Gaseous hydrogen chloride was bubbled through a suspension of the resin in DCM ** Completeness of reaction was determined by a negative ninhydrin test			

Benzotriazolyl esters were used as the activated esters throughout the synthesis. These were prepared from the corresponding protected amino acids by reaction with 1-hydroxybenzotriazole (1 eq.) and dicyclohexylcarbodiimide (1 eq.). The quantities used (in relation to the resin substitution capacity) are listed in Table 2.

Table 2

Cycle no.	Amino acid derivative	Molar excess
1	Boc-Pro-OH	1.8
2	Boc-Tyr(Bzl)-OH	1.8
3	Boc-Trp(CHO)-OH	1.8
4	Boc-Gly-OH	1.8
5	Boc-His(Bom)-OH	1.8
6	Boc-ser(Bzl)-OH	2.0
7	Boc-Trp(CHO)-OH	2.0
8	Boc-His(Bom)-OH	2.0
9	pyroGlu-OH	2.0



Following the final coupling, the resin was washed with dichloromethane (3 x 3 L) and dried under reduced pressure at +40°C to constant weight.

Amino acid analysis: Consistent with proposed sequence

1B. Cleavage and deprotection

pyroGlu-His-Trp-Ser-His-Gly-Trp-Tyr-Pro-Gly-NH<sub>2</sub> (6)

The peptidoresin prepared in Example 1A was placed in a linen bag in a pressure vessel. The vessel was then charged with gaseous ammonia to a final pressure of 4 atm. After 72h the excess ammonia was vented and the resin was extracted with acetic acid (3x100mL) and ethanol (3x100mL). The combined extracts were degassed with nitrogen, 10% palladium-on-carbon was added, and the mixture was stirred under an atmosphere of hydrogen. When the reaction was complete (as judged by HPLC), the mixture was filtered and the filtrate was evaporated. The residue was purified by reverse-phase HPLC to give the title compound.

#### Example 2- Microencapsulation of peptide

Copoly(D,L-lactic acid, glycolic acid) with a lactic acid/glycolic acid ratio of 50/50 is used. To a solution of this polymer (3.7g) in dichloromethane (100mL) in a reaction vessel equipped with a stirrer is added GnRH-II acetate (0.15g, prepared by dissolving the peptide of example 1 in acetic acid and lyophilising the resultant solution). The mixture is stirred at 500revolutions/minute, then silicone oil (Dow Corning 360 Medical Fluid®, 45g) is added over 10 minutes. The mixture is then introduced as a thin jet into caprylic-capric acid-triglyceride (Miglyol® 812, 3.3L) with continuous stirring at 1000revolutions/minute. When addition is complete, stirring is continued for 1 hour, then the microcapsules are collected by filtration, washed twice with isopropanol, and finally dried.

#### Example 3 - Analysis of the effects of GnRH-II and analogues on Osteogenic cell populations *in vitro*.

- (a) Human osteoblasts were isolated from cancerous bone from orthopaedic surgery (Nilsson *et al.*, 1995) according to standard procedures known in the art. The bone

explants were minced into small bone chips and then washed extensively in Dulbecco's modified Eagle's medium (DMEM)/F12 (1:1 Gibco, Paisley, U.K). These osteoblast like cells, Murine osteoblastic MC3T3-E1 cells and human clonal osteosarcoma cell lines MG-63 (non-mineralising) and SaOS-2 (mineralising osteosarcoma) were cultured in DMEM:F12, 1:1 with the addition of 10% fetal calf serum (FCS, Gibco), fungizone (500mg/l), gentamycin sulphate (50mg/l), L-glutamine (2mM) and l-ascorbic acid (100mg/l) in a humidified CO<sub>2</sub> chamber at 37°C.

- (b) Human bone marrow stromal cells were isolated from bone fragments rinsed in phosphate-buffered saline. Bone marrow cells were collected and spun through a column of Ficoll Hypaque (Kimble *et al* J. Clin. Invest. 93 1959-1967, 1994) Cells at the interface were pelleted, counted and seeded into 75cm<sup>2</sup> flasks. The cells were incubated in a humidified CO<sub>2</sub> chamber at 37°C and the medium changed weekly. At confluence, the cells were harvested using trypsin EDTA and re-seeded in  $\alpha$ -minimum essential medium ( $\alpha$ -MEM) supplemented with 10% fetal calf serum (FCS, Gibco), penicillin (100U/ml), streptomycin (100mg/ml), fungizone and L-glutamine (2mM).
- (c) All cells were serum-starved for 48h before addition of GnRH-I and GnRH-II. Cells were placed in DMEM without phenol red (in order to avoid oestrogen-like effects of phenol red) containing 10% charcoal-stripped serum for 48 hours in 12 well plates. Dose dependent effects of GnRH-I and GnRH-II and analogues of the peptides were studied following the addition of peptides at final concentrations ranging from 10<sup>-9</sup> to 10<sup>-6</sup>M. 1mM dibutyryl cAMP was used as a control. The cells were incubated for 24, 48 and 96h with the peptide being replaced every 24 hours.
- (d) TO assess the effects of the peptides on cell proliferation, [<sup>3</sup>H]thymidine was added at 1mCi/ml for an additional 24hours and [<sup>3</sup>H]thymidine incorporation was determined. Radioisotope incorporation was determined using a scintillation counter and the results were calculated as cpm/mg of total protein.
- (e) Expression of osteoblastic differentiation markers was also determined (Tintut Y *et al.* J Biol Chem 273 7547-53, 1998). Total RNA was isolated at several stages

before treatment, at 24, 48, 72 and 96 hours after addition of peptides. Type I procollagen, osteopontin and 28S RNA (used as an internal control) expression was determined by Northern blot analyses. Alkaline phosphatase, matrix GLA protein, osteoclastin and GAPDH (as an internal control) were determined by RT-PCR with specific primers designed for each gene.

The peptides of the invention caused significant effects at concentrations below 100µM.

**Example 4 - Analysis of the effects of GnRH-II and analogues on Osteoclast populations *in vitro*.**

(a) Human clonal cell lines of osteoclast precursors (FLG 29.1) were used as an *in vitro* model of osteoclast differentiation (Gattei V *et al.*, Cell Growth Differ 7 753-63, 1996). In addition, co-cultures of FLG 29.1 and osteoblastic cells (Saos-2) were evaluated for migratory, adhesive, cytochemical, morphological, and biochemical changes. Dose dependent effects of GnRH-I and GnRH-II and analogues of the peptides were studied following addition at final concentrations ranging from 10<sup>-9</sup> to 10<sup>-6</sup>M to FLG 29.1 cultures and to co-cultures. Parathyroid hormone was added as a control. Potentiation (or inhibition) of the differentiation of the preosteoclasts (fusion into large multinucleated elements) and a number of other factors were measured (Orlandini *et al.*, Cell Tissue Res. 281 33-42, 1995). These included:

1. Positive staining for tartrate-resistant acid phosphatase in FLG 29.1 cells
2. A decrease of the alkaline phosphatase activity expressed by Saos-2 cells
3. The appearance of typical ultrastructural features of mature osteoclasts in FLG 29.1 cells
4. The release into the culture medium of granulocyte-macrophage colony stimulating factor.
5. To assess the effects the peptides on cell proliferation, [<sup>3</sup>H]thymidine was added at 1mCi/ml for an additional 24hours and [<sup>3</sup>H]thymidine incorporation was determined as described above.

(b) Bone marrow cells removed from human bone fragments were cultured in the presence of 10nM 1,25-(OH)<sub>2</sub> vitamin D<sub>3</sub> for seven days to generate multinucleated osteoclasts using standard techniques known in the art (Takahashi *et al.*, Endocrinol 122 1473-1482, 1988). The culture medium (α-MEM) was removed and replaced by a fresh phenol red free medium supplemented with antibiotics and 10% charcoal-stripped heat-inactivated FCS containing GnRH-I, GnRH-II or

analogues, and the cultures were maintained for a further 24 hours. Floating cells were harvested and osteoclasts stained for tartrate-resistant acid phosphatase (TRAP) expression, a marker of osteoclast differentiation (Hughes *et al.*, Nat. Med. 2 1132-1135, 1996)

1. Cells were incubated in 0.2M acetate buffer, pH 4.7-5.0, containing tartaric acid and 2% naphthol AS-BI phosphate (dissolved at 20mg/ml in ethylene glycol monomethyl ether) for 15min at 37°C. The cells were then transferred to a second solution consisting of the same buffer and concentration of tartaric acid with 0.1% pararosaniline chloride (hexazotised by mixing with an equal volume of 4% sodium nitrite for 5min at room temperature) for 10min at 37°C. This treatment causes a red cytoplasmic stain in cells expressing TRAP. Harris' hematoxylin was used as a nuclear counterstain.
2. Apoptotic multinucleated osteoclasts were identified by strong expression of TRAP, larger size than accompanying viable TRAP-positive cells. Confirmation of apoptosis was carried out using acridine orange stain. Viable osteoclasts were counted after fixation in 95% ethanol and TRAP hematoxylin staining, and apoptotic osteoclasts were expressed as a percentage of the total number of multinucleated osteoclasts (viable and apoptotic) in each culture well.

The peptides of the invention caused significant effects at concentrations below 100µM.

#### **Example 5- Expression analysis of GnRH mRNA in osteogenic and osteoclast c II populations**

Total RNA was extracted from cells cultured as described above:

1. osteoblast like cells, isolated from cancerous bone
2. murine osteoblastic MC3T3-E1 cells
3. MG-63 (non-mineralising)
4. SaOS-2 (mineralising osteosarcoma)
5. human bone marrow stromal cells
6. human FLG 29.1 osteoclast precursor cells
7. multinucleated osteoclasts generated from bone marrow

Expression of GnRH-I and GnRH-II was determined by RT-PCR using PCR primers outlined in SEQ I.D. No 1-4. The integrity of the cDNA generated was determined by assessing the relative level of actin amplification.

**Example 6 - Effect of GnRH-II on bone mineral density in the ovariectomised rat**

- (a) Female adult (8 weeks old, 200-215g) Sprague Dawley rats were bilaterally ovariectomised (OVX). Animals were kept for 4 weeks post-delivery before commencing treatment. Purina rat chow (1.00% calcium, 0.61% phosphorous) and water were provided ad libitum. Each study consisted of 6 weight-matched groups (n = 8/group).
- (b) Treatment started 4 weeks post-OVX. After 4 weeks, a baseline control OVX group was sacrificed (Group A). The remaining groups were injected once a day with vehicle (Group B), 1 $\mu$ g/kg body weight (Group C), 10 $\mu$ g/kg body weight (Group D), 100 $\mu$ g/kg body weight (Group E) of GnRH-II, and 80 $\mu$ g/kg body weight (Group F) of hPTH(1-34).
- (c) All rats were weighed every fourth day and dosages adjusted for 50g increase in mean group weight. Rats were given alternate subcutaneous injections of calcein (30mg/kg) or tetracyclin (30mg/kg) in 2% sodium bicarbonate-saline, respectively to label mineralization surfaces on days 10, 19 and 26, following treatment with drug. Bone mineral density was assessed by dual energy x-ray absorptometry-DEXA. On day 28 serum calcium levels were determined by colorimetric assay using a commercial kit.
- (d) Success of OVX was confirmed at necropsy by failure to detect ovarian tissue and by observation of marked atrophy of the uterine horns. Both legs were disarticulated at the hip. The left tibia and femur were cleaned of excess muscle and soft tissue and placed in 70% ethanol. The anterior eminence of the right tibia metaphysis was shaved with a razor blade, barely exposing bone marrow. Both right femur and tibia were then placed in 10% phosphate-buffered formalin for 24h and transferred to 70% ethanol.

Ovariectomised animals treated daily with 10 and 100 $\mu$ g/kg of GnRH-II and 80 $\mu$ g/kg PTH for 28days have pronounced hypercalcemia. Results are shown in Figure 1.

**Example 7 - Cellular localisation of GnRH-II in paraffin sections of normal rat bone and human bone.**

- (a) Frozen and/or paraffin-embedded human and rat bone sections were fixed for 3-36h depending on size (3-5h at room temperature, then approx 24h at 4°C) and then soaked in 0.1M Tris + 5 % EDTA (12.11g + 50g EDTA) pH 7.3 until decalcified.
- (b) Sections were then processed for antibody staining (rabbit polyclonal anti-GnRH-II antibody) using standard techniques.

Staining for GnRH-II was observed in platelets, megakaryocytes at the growth plate (especially proliferating chondrocytes). Some staining was also seen in the bone-forming cells particularly in active osteoblasts as well as new osteoid.

Example 1 demonstrates the preparation of the peptides of the invention, which can then be formulated as illustrated in Example 2. Examples 3 to 7 demonstrate the biological activity of the peptides of interest. The scope of the invention is not intended to be limited in any way by these Examples. In particular, it will be realised that variety of controlled release formulations of these peptides can be prepared by varying the polymer and/or the physical nature of the combination of the peptide and polymer. However, these variations give formulations with equivalent biological properties, and are intended to be within the scope of the invention as defined in the following Claims.

SEQ I.D. Nos. 1 to 4 referred to in Example 5 are as follows :

CTG	CAG	CTG	CCT	GAA	GGA	C	(1)
GGG	CGG	GGC	GGG	GCT	CTC	G	(2)
ATT	CTA	CTG	ACT	TGG	TGC	GTG	(3)
GGA	ATA	TGT	GCA	ACT	TGG	TGT	(4)